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## The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows

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### ABSTRACT

The objective of this prospective study was to determine associations between occurrence and severity of clinical (CM) and subclinical mastitis (SM) during a defined breeding risk period (BRP, 3 d before to 32 d after artificial insemination) on pregnancies per artificial insemination at first service (P/AI1). Dairy cows ( $n = 3,144$ ) from 4 Wisconsin herds were categorized based on the occurrence of one or more CM or SM events during and before the BRP: (1) healthy, (2) mastitis before BRP, (3) SM during BRP, (4) chronic SM, (5) CM during BRP, or (6) chronic CM. Clinical mastitis cases were categorized based on etiology (gram-negative, gram-positive, and no growth) and severity (mild, moderate, or severe). Compared with healthy cows, the odds of pregnancy were 0.56, 0.67, and 0.75 for cows experiencing chronic CM, CM, or SM during the BRP, respectively. The occurrence of chronic SM was not associated with reduced probability of P/AI1. Compared with healthy cows, the odds of pregnancy were 0.71 and 0.54 for cows experiencing mild or moderate-severe cases of CM during the BRP, respectively. The odds of pregnancy for cows experiencing CM caused by gram-negative or gram-positive bacteria during the BRP were 0.47 and 0.59, respectively. The occurrence of CM that resulted in no growth of bacteria in cultured milk samples was not associated with reductions in P/AI1. Regardless of etiology, microbiologically positive cases of CM with moderate or severe symptoms were associated with substantial reductions in P/AI1. Etiology, severity, and timing of CM were associated with decreases in the probability of pregnancy at first artificial insemination. Severity of the case was more important than etiology; however, regardless of severity, microbiologically negative cases were not associated with reduced probability of pregnancy.

**Key words:** mastitis, dairy, fertility, animal reproduction

### INTRODUCTION

Reproductive efficiency is one of the most important factors associated with dairy farm profitability and is negatively affected by diseases such as mastitis. The occurrence of mastitis has been associated with increased days to first AI (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004), increased services per conception (Schrick et al., 2001; Santos et al., 2004; Ahmadzadeh et al., 2009), increased days open (Schrick et al., 2001; Santos et al., 2004; Ahmadzadeh et al., 2009), increased incidence of pregnancy loss (Risco et al., 1999; Santos et al., 2004), and decreased pregnancies per AI at first AI (P/AI1; Santos et al., 2004).

Mastitis is a bacterial infection of the mammary gland that is recognized based on the inflammatory response to infection. Subclinical mastitis (SM) is defined as an IMI that results in an influx of inflammatory cells (somatic cells) and is usually detected based on increased SCC of milk (Ruegg and Erskine, 2014). Clinical mastitis (CM) is defined as an IMI that results in production of abnormal milk with or without abnormalities in the mammary gland or systemic symptoms (Pinzón-Sánchez and Ruegg, 2011; Ruegg and Erskine, 2014). The occurrence of both subclinical (Schrick et al., 2001; Lavon et al., 2011a,b; Hudson et al., 2012) and clinical (Moore et al., 1991; Santos et al., 2004; Hudson et al., 2012) mastitis has been associated with reduced reproductive performance. The occurrence of the mastitis event relative to insemination mediates the effect of mastitis on pregnancy outcomes (Barker et al., 1998; Santos et al., 2004). The most detrimental effects of mastitis on reproductive performance were observed when CM occurred near the time of AI (Hertl et al., 2010; Hudson et al., 2012) or during the interval between AI and first pregnancy diagnosis (Barker et al., 1998; Santos et al., 2004). The occurrence of SM during the same interval also negatively affects conception (Schrick et al., 2001; Lavon et al., 2011a; Hudson

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et al., 2012); however, the effects of repeated mastitis events occurring near the time of AI (either before or during AI) and between AI and the first pregnancy diagnosis have not been well described.

Mastitis is caused by a variety of pathogens, and the reported effect of specific pathogens on reproductive performance has been inconsistent. Several studies have reported similar reductions in reproductive performance for mastitis caused by gram-positive and gram-negative pathogens (Barker et al., 1998; Schrick et al., 2001; Santos et al., 2004). In contrast, Moore et al. (1991) and Hertl et al. (2010) both reported a greater reduction in reproductive outcomes when mastitis was caused by gram-negative compared with gram-positive organisms. Pathogen specific differences in reproductive performance have been hypothesized to result from differences in the immune response to IMI caused by different pathogens (Schukken et al., 2011). However, severity of CM symptoms is associated with etiology (Oliveira and Ruegg, 2014) and no studies have evaluated the effect of severity of CM on reproductive outcomes. The objective of this study was to describe associations between the occurrence and severity of CM and SM occurring during a defined risk period with P/AI while accounting for etiology and previous mastitis events.

## MATERIALS AND METHODS

### *Herds and Cow Enrollment Criteria*

A total of 3,277 cows from 4 Wisconsin dairy herds were eligible to be enrolled in a prospective cohort study between May 2011 and November 2013. Eligibility criteria for farms included participation in monthly DHIA testing that included individual cow SCC data, use of DairyComp 305 for herd records, use of a con-

sistent breeding program throughout the trial period, and administration of intramammary dry cow therapy to all quarters of all cows. Milking technicians were requested to use a complete milking routine that included forestripping for detection of clinical mastitis. All cows eligible to receive a first AI during the enrollment period on each farm were eligible for enrollment in the study, and each cow could be enrolled once. Cows not eligible for AI were excluded. Cows were also excluded if pregnancy status after AI was not available (due to culling or death) or if they did not have complete mastitis records (defined as the following data recorded for each case of CM: quarter affected, date identified, and severity, and for SM: individual cow SCC near AI). Cows were followed until the outcome of the first pregnancy diagnosis was ascertained.

Of a total of 4,378 lactating cows that were present on the 4 enrolled farms, 3,277 were eligible for first AI during the enrollment period (Table 1). Of eligible cows, 113 did not receive a first AI and were not enrolled (Table 1). Cows ( $n = 889$ ) were enrolled on farm A between May to November 2011; cows ( $n = 981$ ) were enrolled on farm B between March 2012 until February 2013; cows ( $n = 735$ ) were enrolled on farm C between May 2012 until July 2013; and cows ( $n = 559$ ) were enrolled on farm D between March to November 2013. Of the enrolled cows ( $n = 3,164$ ), severity scores were not recorded for 20 cows, leaving 3,144 cows with complete records for statistical analysis (Table 1).

All procedures were approved by the Animal Care and Use Committee for the College of Agricultural and Life Sciences of the University of Wisconsin–Madison.

### *Data Collection*

During the data collection period (from calving to first pregnancy diagnosis), the occurrence of SM was

**Table 1.** Descriptive characteristics of enrolled cows ( $n = 3,164$ ) from 4 Wisconsin dairy herds

Farm	Milking cows per herd (n)	Cows eligible for the study <sup>1</sup>	Cows enrolled in the study <sup>2</sup>	Cows used for analysis <sup>3</sup>	P/AI <sup>4</sup> (%)	Use of Synch <sup>5</sup> (%)	Milk yield <sup>6</sup> (kg)	SCC <sup>7</sup> (cells per mL)
A	1,429	913	889	888	39.0 <sup>a</sup>	93.9 <sup>c</sup>	46.1 <sup>b</sup>	51,823 <sup>b</sup>
B	1,382	1,017	981	965	44.7 <sup>b</sup>	87.6 <sup>b</sup>	46.0 <sup>b</sup>	47,492 <sup>ab</sup>
C	817	761	735	734	48.7 <sup>b</sup>	99.5 <sup>d</sup>	48.6 <sup>c</sup>	44,723 <sup>a</sup>
D	750	586	559	557	38.6 <sup>a</sup>	57.6 <sup>a</sup>	43.0 <sup>a</sup>	72,639 <sup>c</sup>
Total	4,378	3,277	3,164	3,144	42.9	86.7	46.1	51,788

<sup>a-d</sup>Within a column among farms, means (ANOVA) or proportions (logistic regression) with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Cows eligible for AI at first service.

<sup>2</sup>Cows submitted for first service.

<sup>3</sup>Enrolled cows that had complete records of the severity of CM and used for statistical analysis.

<sup>4</sup>Pregnancies per artificial insemination at first AI (P/AI).

<sup>5</sup>Percentage of cows submitted for first AI using a hormonal synchronization protocol.

<sup>6</sup>Milk yield from 3 d before to 32 d after first AI.

<sup>7</sup>SCC from 3 d before to 32 d after first AI. Somatic cell count values were  $\log_{10}$ -transformed, then back-transformed to SCC per milliliter.

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